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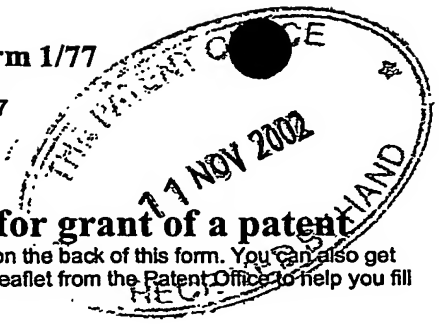

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12NOV02 E762648-1 003312
P01/7700 0.00-0226274.9

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Patents ADP number (if you know it) 8S0392200|

If the applicant is a corporate body, give the country/state of its incorporation United Kingdom

4. Title of the invention METERED DOSE INHALATION PREPARATIONS

5. Name of your agent (if you have one)
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METERED DOSE INHALATION PREPARATIONS

The present invention relates to glycosidically stabilised preparations of therapeutic materials for use in metered dose inhalation devices, and methods for their preparation.

Pulmonary delivery has been employed for many years for drugs intended to have localised, rather than systemic, effects. Essentially, there are three types of device available for pulmonary delivery, and these are nebulisers, metered dose inhalers (MDI) and dry powder inhalers (DPI). Each of these has its benefits and its drawbacks.

Nebulisers are particularly effective in the administration of aqueous formulations of drug to non-ambulatory patients. Drug solution is converted into microdroplets which are inhaled by the patient, these microdroplets providing the facility to deliver the drug in a variety of dose volumes, ranging from several milligrams to grams. In addition, the very small droplets can be deposited effectively in the smaller airways, compared to those particles produced by either metered dose inhalers or dry powder inhalers. However, nebulisers are generally large, and unsuitable for ambulatory use. In addition, there is a problem with the potential instability of drugs in aqueous solution, as well as during the process of nebulisation, and reproducible dosing can be difficult.

MDI's are the most widely used pharmaceutical inhalation devices. The formulations used in these devices routinely comprise drug, propellants, and stabilising excipients. In general, the drug is formulated together with the excipients and then combined with the propellants, under pressure. Fine respirable particles of drug are then produced as a consequence of the break-up of droplets expelled from the device under pressure, followed by extremely rapid evaporation of the propellants. The amount of drug is controlled by delivering a pre-metered volume of propellant/drug mixture.

The suitability of MDI's to deliver peptide and protein pharmaceutical has not been well established, and there are serious concerns for the physical and chemical stability of formulated proteins and peptide particles in propellant solutions. For both

this reason and the ability to deliver in more substantial quantities, dry powder inhalation devices are generally preferred for pulmonary delivery of proteins and peptides.

Dry powder inhalation devices dispense powder solely by the force of the patient's inspiration, or by an external source with which the device is equipped. As the drug can be formulated simply as fine drug particles, or together with a coarse carrier, such as lactose or glucose, there is less likelihood of long term stability problems, compared with MDI solutions. Since it is also possible to deliver relatively large amounts of drug by DPI's, then current studies involving the systemic delivery of peptides and proteins have generally employed DPI's rather than MDI's.

However, unlike MDI's, the ergonomics of DPI's are manufacture-dependent and, as a result, this can cause confusion amongst patients, which may lead to poor efficacy of therapy. In one study, 40% of patients who had been taught how to use a Turbuhaler®, and who had used it for between 8 months to 8 years, used it so poorly that it was unlikely that the patients were obtaining any therapeutic benefit from the inhaled drugs (Terzano, 2001).

Where the amount to be delivered is not an issue, then the benefits of using DPI's over MDI's appears to be equivocal. In recent studies, there was no evidence that DPI's were any more effective in delivering corticosteroids and ß-2 agonist bronchodilators in asthma than MDI's.

In addition, the aerodynamic performance of MDI and DPI devices containing the same glucocorticoid was compared *in vitro*, and it was established that the fine particle mass (FPM) delivered by the DPI was flow rate dependent and significantly lower than that achieved using the MDI.

Thus, the primary advantage of DPI's lies in their ability to dispense large quantities of drug from a stable, powder formulation. By contrast, MDI's are able to dispense formulation in a more controlled, and more effective manner, but formulation instability as a function of storage time can be a problem. This can manifest itself either by chemical degradation of the drug or, alternatively, as a change in physical stability of

the formulation, which can lead to particle aggregation and a lowering in the respirable fraction, or both.

MDI's are propellant-based delivery systems which, until recently, relied on the use of chlorofluorocarbons, or CFC's [trichlorofluoromethane (CFC-11) dichlorofluoromethane (CFC-12) and 1,2-dichlorotetrafluoroethane (CFC-114)], in varying ratios, as the principal component of the formulation. With the universal phased withdrawal of the use of CFC's, the only two propellants currently approved for inhalation are tetrafluoroethane (HFA-134a) and heptafluoropropane (HFA-227). Both of these hydrofluoroalkanes have boiling points substantially below 0°C, unlike CFC-11 (23.8°C), so that formulation design is further complicated. In addition, the HFA's have poor solvency for those surfactants commonly employed as excipients in CFC-based MDI's, so that a co-solvent generally needs to be included. Surfactants are required in order to maintain physical stability, thereby ensuring aerodynamic performance, and to act as a valve lubricant. However, surfactants, which are usually presented as dissolved excipients, can lead to the production of aerosolised aggregates during the evaporation of sprayed droplets, and such detrimental effects can increase with increasing suspension concentration. Thus, if possible, it would be desirable to avoid the use of both surfactants and co-solvents.

In developing MDI or DPI formulations of peptides and proteins, it is of primary importance to maintain the stability of peptide and protein drugs during processing and storage, as well as ensuring the efficiency and reproducibility of the deposition of drug particles during use by the patient. Particular considerations for MDI's include the production of particles with controlled particle size and stability, and compatibility between propellants and the proteins and peptides. Such factors ensure that the suspension and biological stability can be maintained over the required shelf life.

Technical considerations for DPI's, apart from the production of particles identical to MDI's, can further include moisture control, reproducible powder filling, unit based packaging, particle dispersibility and development of desirable inhalers, thereby rendering their satisfactory development and quality control often more difficult than MDI's.

The compatibility of HFA propellants with protein powders has been investigated in a number of previous studies. For example Quinn *et al.* found that protein MDI formulations retained the biological activity of tested peptides and proteins, such as calcitonin and deoxyribonuclease I [Int. J. Pharm. (1999) 186, 31-41] found that the conformation of lysozyme underwent no change in the presence of HFA-134a as analysed by Fourier transform Raman spectroscopy. In other studies, workers from 3M Limited found that protein MDI formulations retained the biological activity of tested peptides and proteins, such as calcitonin and deoxyribonuclease I [Koppenhagen, *et al.* (2001), Evaluation of pressurised metered dose inhalers for pulmonary delivery of proteins and peptides, www.3m.com/us/healthcare/manufacturers/dds/pdf/pub_2001_09_koppenhagen.pdf; Oliver, *et al.*, (2000), Initial assessment of a protein formulated in the pressurized metered dose inhalers for pulmonary delivery, www.3m.com/us/healthcare/manufacturers/dds/pdf/pub_2000_05_oliver.pdf]. Other work also suggests that MDI protein formulations might be efficient in terms of aerodynamic performance and reproducibility, in terms of dosimetry.

Accordingly, if it were possible to provide MDI formulations of protein having both suitable chemical and physical stability during manufacture and storage, then MDI's would have substantial advantages over DPI's for the delivery of appropriate therapeutic substances.

Surprisingly, we have now found that glycosidically stabilised complex drugs, or macromolecules, such as proteins and peptides, have substantially greater stability in the presence of HFA's, when formulated with polyhydroxylated polyalkenes, such as PVA.

Accordingly, in a first aspect, the present invention provides a formulation of a therapeutic substance suitable for delivery to a patient by a metered dose inhalation device, the substance being in association with a stabilising amount of a glycoside and being formulated in one or more propellants, characterised in that the therapeutic substance is first prepared as a substantially dry powder in the presence of a polyhydroxylated polyalkene, prior to formulation with propellant.

Therapeutic substances are generally any substance suitable for administration *via* an MDI device for therapeutic purposes, whether for prophylaxis or treatment. In general, therapeutic substances suitable for use in the formulations of the present invention are advantageously larger, organic molecules, such as peptides and proteins and may include therapeutic glycosides and steroids, for example. Such molecules may have substantial stability in the presence of HFA's, but the majority of peptides and proteins are not conformationally stable over long periods, and may lose activity, or physical stability, or often both. This loss of activity arises not only through degeneration of the peptide or protein, but also from clumping of the suspended formulation particles, which serves to reduce the fine particle mass critical for the treatment of the patient.

Such large organic molecules may be stabilised by the presence of suitable glycosidic compounds, particularly the lower oligosaccharides. The composition of the oligosaccharide is not critical to the present invention, and the molecule may comprise a mixture of furanosyl residues, pyranosyl residues, and straight chain elements. For example, sucrose comprises a furanosyl and a pyranosyl residue, whilst mannitol comprises a pyranosyl residue and a straight chain element. Other suitable disaccharides include lactose, isomaltose, cellobiose, maltose and trehalose, of which trehalose is preferred. Other suitable oligosaccharides include raffinose, melezitose and stachyose. It will be appreciated that the present invention envisages the use of any of these, or other, oligosaccharides either individually or as mixtures.

Other glycosidic compounds that may be used may generally be described as polyols, and include such compounds as mannitol, xylitol, sorbitol, maltitol, isomalt and lactitol.

Suitable amounts of the polyol compounds are, very approximately, on parity with the therapeutic substance, by weight. More generally, the amount of polyols may vary between about 30% and 400% by weight of the therapeutic substance. The terms "polyol" and "glycoside" are used interchangeably herein.

It will be appreciated that the polyols are preferably simply carbohydrate compounds, but the present invention also includes derivatives thereof, such as halo-

substituted polyols and blocked polyols, such as the acetates. In particular, the present invention also extends to the glucuronides.

It is an advantage of the present invention that, by combination with a glycoside and a suitably substituted polyalkene, the therapeutic substances are now able to be provided in formulations which are stable, even in the presence of haloalkane propellants. It is a particular advantage that such stability is demonstrated in the presence of HFA's, but it will be appreciated that such stability is also demonstrated in the presence of other propellants, such as CFC's, and alkanes, such as butane and propane.

Preferred propellants are the haloalkanes, and it is preferably envisaged that HFA's are used as propellants for MDI's in formulations of the present invention. However, it will be appreciated that the invention also extends to the use of CFC's and other alkanes, for example. The backbone of the propellant will generally be an alkane, whether substituted or unsubstituted, and may be straight or branched. Where branched, it is preferred that there only be one branch. Straight chains of the lower alkanes are preferred, especially C_{2-4}.

The preferred HFA's for use in the present invention are HFA-134a and HFA-227, with HFA-134a being preferred.

Suitable polyhydroxylated polyalkenes for use in the present invention include PVA, PVAc (polyvinylalcohol and polyvinylacetate, respectively) and poly(vinyl alcohol-co-ethylene). These polyalkene compounds may be substituted as described above for the polyols, but it is generally preferred that they be the simple compounds, rather than their derivatives.

The size of the polyalkene compounds is not critical to the present invention, and PVA may range from a molecular weight of 9kDa through to about 150kDa. In addition, PVA is generally prepared by the hydrolysis of PVAc, and the level of hydrolysis may be as low as about 70% through to substantially complete hydrolysis, such as 98% or higher. High levels of hydrolysis correspond to lower levels of hydrophilicity/higher levels of hydrophobicity, which can affect the formulations of the

present invention. It is generally preferred that the level of hydrolysis be in the region of 75 to 90%, with a level of about 80% being a preferred embodiment.

The molecular weight of the PVA is also not critical to the present invention, but higher molecular weights can generally prove slightly more difficult for formulation, and it is generally preferred to employ lower molecular weight polyalkenes, generally in the region of between about 5kDa and 50kDa, with a range of about 9kDa to 30kDa being more preferred. Where PVA is used as the sole polyalkene, then a preferred molecular weight is in the region of 10kDa. It will be appreciated that molecular weights for the polyalkenes are necessarily highly approximate, as the methods for their preparation necessarily result in a spread of molecular sizes.

Suitable amounts of polyalkenes range from about 5% to about 200% by weight of the therapeutic substance, although there is little advantage to be seen in the provision of large amounts of the polyalkene. In general, a suitable amount of polyalkene is between about 10% and about 50% by weight of the therapeutic substance with a range of about 20% to about 40% being preferred.

Prior to formulation with the haloalkane propellant, it is preferred to blend the therapeutic agent with the polyol and polyalkene in an aqueous vehicle, prior to drying. The aqueous vehicle may be any suitable, and will typically be selected from saline or a suitable buffer such as phosphate buffered saline (PBS), although deionised water may also be used, if desired.

The sequence of addition to the aqueous vehicle is not critical to the present invention. Likewise the powdered products resulting from the drying of the aqueous preparation may be achieved by any suitable drying process, including freeze-drying, spray-drying, spray-freeze-drying, supercritical drying, co-precipitation and air-drying. Of these, spray-drying and spray-freeze-drying are preferred, as these result in fine powders which generally require no further processing. However, if required, the dried products may be further processed to reduce the size of the resulting particles to an appropriate level. In particular, it is preferred that the aerodynamic diameter of the particles of the powder used in the formulations of the present invention is between about 1μm and 12μm, more particularly between about 1μm and 10μm. It is further

preferred that the median aerodynamic diameter is in the range of about 2.5 to 3.5μm, and is preferably between 2.5 and 3μm.

The dried powder is then brought into contact with the propellants under conditions suitable for storing in a reservoir useful in an MDI.

It is a particular advantage of the present invention that the stability of the particles prepared as described above is considerably greater than anything provided in the art. In addition, while not precluded by the present invention, it is a further advantage that it is not necessary to use any further co-solvents or surfactants in the formulations of the present invention, thereby avoiding the problem of coagulation of the particles in the propellant mixture, with time. Thus, formulations of the present invention provide long-term stability of activity of the therapeutic substance, as well as ensuring consistency of dosing with time.

It will be appreciated that the present invention further provides a powdered formulation of a therapeutic agent, a glycoside and a polyhydroxylated polyalkene suitable for incorporation with a haloalkane propellant for dispensing from a metered dose inhaler.

The present invention further provides a metered dose inhalation device provided with a reservoir comprising a haloalkane propellant prepared with a therapeutic substance, a glycoside and a polyhydroxylated polyalkene.

Doses delivered by the MDI's of the present invention will be readily determined by those skilled in the art and as appropriate to the condition to be treated. In general, doses will vary with the size and age of the patient and can be readily determined by calculating the concentration of the active ingredient in the propellant preparation.

Suitable macromolecular compounds for use as therapeutic agents include antibodies, interferon, such as α-interferon, β-interferon and γ-interferon, enzymes such as proteases and ribonucleases, especially DNase I, hormones, such as insulin, LHRH, granulocyte-colony stimulating factor, calcitonin, heparin, human growth hormone, leuprolide acetate and parathyroid hormone, protease inhibitors, such as α1 antitrypsin, as well as beclomethasone dipropionate. Other large and/or complex molecules or

structures, such as virus particles, for example, may also be incorporated in MDI formulations, in accordance with the present invention.

The present invention will now be further illustrated by the following, non-limiting examples.

EXAMPLE 1

Compositions And Spray-Drying Process For Preparing Particles

Particles suitable for admixture with a propellant mixture were prepared as follows. Buffer phosphate salts (ACS reagent grade), sodium chloride, PVA (MW, 9,000-10,000), sucrose, trehalose, lysozyme, and catalase were purchased from Sigma-Aldrich Co.

Enzymes and excipients were dissolved in buffer or saline and spray-dried using a Model 190 Büchi mini spray-dryer. The solutions employed to dissolve lysozyme and catalase were 5 mM sodium phosphate buffer (pH 6.2) and 5 mM potassium phosphate buffer (pH 7.0), respectively, and the enzyme concentrations were maintained at 5 mg/ml. The compositions of the spray-dried formulations are shown in Table 1, below.

Table 1

The compositions and designations of spray-dried enzyme formulations.

Formulation	Composition
LO1:0	Lysozyme 5 mg/ml
LS1:1	Lysozyme 5 mg/ml+sucrose 5 mg/ml
LT1:1	Lysozyme 5 mg/ml+trehalose 5 mg/ml
LPT5:0.5:5.5	Lysozyme 5 mg/ml + PVA 0.5 mg/ml + trehalose 5.5 mg/ml
LPT5:1:6	Lysozyme 5 mg/ml + PVA 1 mg/ml + trehalose 6 mg/ml
LPT5:2:7	Lysozyme 5 mg/ml + PVA 2 mg/ml + trehalose 7 mg/ml
LPT1:1:2	Lysozyme 5 mg/ml + PVA 5 mg/ml + trehalose 10 mg/ml
CO1:0	Catalase 5 mg/ml
CS1:1	Catalase 5 mg/ml + sucrose 5 mg/ml
CT1:1	Catalase 5 mg/ml + trehalose 5 mg/ml
CPT5:1:6	Catalase 5 mg/ml + PVA 1 mg/ml + trehalose 6 mg/ml

The feed solution was pumped peristaltically through a silicone tube (3 mm) to a two fluid nozzle (0.5 mm) head used to atomise the fluid. Cooling water (0°C) was circulated through the jacket around the nozzle at a rate of about 36 ml/min. The processing parameters were: a feed rate of 3 ml/min; an atomising air-flow rate of 700 l/h; and an inlet temperature of 95°C. Outlet temperatures were found to range from 65 to 69°C. The solution volume employed to produce each spray-drying batch was 100 ml and each process lasted ~34 min. The protein powders were collected in a collection jar, after all the feed solution had been processed, but without allowing time for the powder to cool to room temperature, the material was transferred to a 7 ml vial, which was immediately sealed by capping. This vial was then transferred to a freezer (-20°C) for storage.

EXAMPLE 2

Relative Enzyme Activity Remaining After Spray-Drying

The activity of the enzyme in each formulation is shown in Table 2. Spray-dried lysozyme was found to retain about 87% of the original activity, whilst those formulations containing excipients appeared to maintain almost the full activity of the original enzyme. Inactivation of catalase upon spray-drying was found to be about 55% of the initial activity, but the loss of activity was reduced to about 7% when either sucrose or trehalose was included, and almost full activity was recovered when a PVA-trehalose mixture was included in the formulation.

Table 2

Recovered biological activity in spray-dried lysozyme and catalase particles (Mean±SD, n=3).

Formulation	Relative activity (%)	Formulation	Relative activity (%)
LO1:0	87.2±2.1	LPT1:1:2	95.9±3.0
LS1:1	97.2±3.0	CO1:0	54.4±4.1
LT1:1	96.9±3.2	CS1:1	92.3±2.8
LPT5:0.5:5.5	100.4±2.2	CT1:1	93.38±2.1
LPT5:1:6	97.1±3.7	CPT5:1:6	99.8±2.8
LPT5:2:7	97.3±4.8		

EXAMPLE 3

Geometric Particle Size Of Spray-Dried Particles

The particle size as well as size distribution of the spray-dried protein particles are shown in Table 3. The volume median diameters (VMD) of all spray-dried particles were found to be between 2.48 and 3.43 μm. In addition, the span of particle size

distribution was found to be between 0.77 and 1.18, which indicates that all the powders exhibited a relatively high degree of monodispersity, whilst the upper limit of the size range of the particles appeared to be ≤12.5 μm.

Table 3

Particle size and distribution of spray-dried lysozyme and catalase formulations.

Formulation	Median diameter (μm)	Span	Size range (μm)
LO1:0	3.09	0.77	1.22-7.49
LS1:1	3.43	0.92	1.22-12.5
LT1:1	3.31	0.91	1.22-11.6
LPT5:0.5:5.5	2.48	1.15	1.22-7.49
LPT5:1:6	2.67	1.03	1.22-9.31
LPT5:2:7	2.78	1.18	1.22-10.0
LPT1:1:2	2.89	1.13	1.22-10.8
CO1:0	3.12	1.18	1.22-10.0
CS1:1	2.95	1.04	1.22-9.31
CT1:1	2.96	1.15	1.22-10.0
CPT5:1:6	2.77	1.33	1.22-10.0

EXAMPLE 4

The Effect Of HFA On The Biological Activity Of Spray-Dried Enzymes

For spray-dried lysozyme and catalase in the presence of sucrose, trehalose or a trehalose-PVA mixture, there appeared to be no detectable reduction in activity after being stored in a HFA based-MDI canister up to 26 weeks, whilst the activity of spray-dried catalase alone was found to be reduced to ~20% within 12 weeks (Table 4).

Table 4

The retained activity of HFA based MDI-formulated lysozyme and catalase particles relative to the corresponding control powders after storage for 12 weeks at room temperature.

Formulation	Activity of MDI-formulated enzyme at Week-1 (%)	Activity of MDI-formulated enzyme at Week-12 (%)
LO1:0	99.0	98.1
LPT5:0.5:5.5	96.8	100.0
LPT5:1:6	102.9	95.3
LPT5:2:7	98.9	99.0
LPT1:1:2	97.6	93.0
CO1:0	98.9±2.7	20.8±6.8
CPT5:1:6	101.1±4.7	98.2±1.8

EXAMPLE 5

Delivery Of MDI-Formulated Enzyme Particles

The mean dose of protein delivery through the valve of each of six formulations, each of which comprised 3 different batches, was found to be between 1395 and 1524 μg per 20 actuations. The variation coefficients between batches of each formulation and between formulations were less than 10% (Table 5). These results indicated that the uniformity of MDI formulations was satisfactory and was independent of batches and formulations. The mean recovery of protein from the 6 formulations was found to range from 94.3 to 101.6, with a mean of 97.0±6.4% (n=18).

Table 5

The dose delivery through the valve and the recovery after deposition of spray-dried enzyme MDI suspension formulations at the first week after preparation (Mean±SD, n=3).

Formulation	Dose delivery through the valve (μg of protein 20 actuation)	Recovery (%)
LS1:1	1438.0±126.0	94.3±3.2
LT1:1	1446.5±89.0	98.6±8.6
CS1:1	1524.0±33.6	94.3±4.4
CT1:1	1395.7±70.2	101.6±7.7

EXAMPLE 6

Deposition Of MDI-Formulated Lysozyme Particles

The *in vitro* deposition performance of MDI-formulated spray-dried lysozyme particles is shown in Table 6. For the formulation prepared using spray-dried lysozyme alone, the protein fractions recovered from the device, stage 1 and stage 2 were found to be about 14.6, 34.9 and 50.5% respectively, during the first week after preparation. After storage at room temperature for up to 12 weeks, the stage 2 fraction significantly decreased to 42.7% whilst the stage 1 fraction increased to 42% of the recovered dose ($p<0.05$, one tailed student t-test, Table 6).

When lysozyme was stabilised using either sucrose or trehalose as excipient during spray-drying, the aerodynamic properties of the resultant MDI formulations were significantly affected ($p<0.05$, two tailed student t-test). At the first week after manufacture, the stage 2 fraction of MDI formulation LS 1:1 appeared to decrease to 27.2% whilst the fraction recovered from the device and stage 1 increased to 21.7 and 51.3% respectively. After 6 weeks storage at room temperature, the stage 2 fraction decreased significantly to about 8% ($p<0.05$, two tailed student t-test). However, with

further storage for up to 26 weeks, there appeared to be no more reduction in the stage 2 fraction. The MDI formulated LT1:1 particles displayed a similar aerodynamic performance to the LS1:1 formulations at the first week after preparation. However, the storage suspension stability of the former proved to be significantly better than the latter ($p<0.05$, paired student t-test). Nonetheless, the fine particle fraction (stage 2 fraction) of LT1:1 MDI formulation was susceptible to decrease as a function of storage time. After stored for 26 weeks, the fine particle fraction significantly decreased to 12.7% ($p<0.05$, two tailed student t-test) whilst the stage 1 fraction increased to 61.4% of the recovered protein.

When lysozyme was spray-dried in the presence of PVA-trehalose mixture, the resultant MDI formulations appeared to have significantly better aerosol performance than those formulated using either trehalose or sucrose alone in combination with the enzyme ($p<0.05$, two tailed student t-test, Table 6). The fine particle fraction of MDI formulations containing PVA were found to range from 47.1 to 52.7% at the first week after preparation. The stability of aerodynamic properties was found to depend upon the PVA content in the spray-dried particles. After storage for 12 weeks at room temperature, the formulation (LPT5:0.5:5.5) containing the lowest PVA content was found to emit a insignificantly decreased fine particle fraction of 42.8%, in comparison to the 48.3% obtained during week-1 ($P>0.05$, one tailed student t-test). The other formulations containing a higher ratio of PVA content in the formulations appeared to retain a constant fine particle fraction over the 12 week storage period. All the PVA containing MDI formulations displayed a significantly better storage suspension stability, in terms of fine particle fraction, than either the MDI LS1:1 or LT1:1 formulations ($p<0.05$, paired student t-test).

Table 6

The aerosol performance of HFA based MDI lysozyme formulations as evaluated by a twin stage impinger after storage up to 12 weeks (Mean±SD, n=3).

Formulation	Stage	Fraction at week-1 (%)	Fraction at week-6 (%)	Fraction at week-12 (%)
LO1:0	Device	14.59±3.21	15.41±2.55	15.29±3.11
	Stage 1	34.90±2.75	34.27±1.85	42.01±2.89
	Stage 2	50.51±3.76	50.32±0.81	42.69±3.41
LPT5:0.5:5.5	Device	15.97±2.9	10.92±1.05	15.12±3.56
	Stage 1	37.54±3.14	40.88±2.73	42.07±2.51
	Stage 2	48.27±3.3	48.20±3.65	42.80±3.89
LPT5:1:6	Device	15.20±1.10	15.30±2.06	16.41±2.78
	Stage 1	39.33±6.08	39.39±1.97	38.72±4.10
	Stage 2	47.14±9.12	45.30±0.30	44.87±3.67
LPT5:2:7	Device	18.29±2.23	10.87±1.03	13.49±1.32
	Stage 1	33.18±6.60	41.65±1.76	37.92±2.87
	Stage 2	48.54±4.46	49.11±3.25	48.59±3.2
LPT1:1:2	Device	19.32±2.97	13.35±1.70	12.47±3.21
	Stage 1	30.57±1.49	33.30±4.38	32.10±2.98
	Stage 2	52.73±1.97	54.42±3.10	55.44±3.70

EXAMPLE 7

Deposition Of MDI-Formulated Catalase Particles

The *in vitro* deposition performance of MDI catalase formulations is shown in Table 7. For the MDI-formulated spray-dried catalase without any excipient, the protein fractions recovered form device, stage 1 and stage 2 were found to be about 23.7, 43.3 and 33.0% respectively, during the first week after preparation. However, after the same formulations had been stored for 6 weeks at room temperature, the stage 2 fraction was found to decrease drastically to almost 0% with about 89% of particles being deposited in stage 1 (Table 7).

Table 7

The aerosol performance of HFA based MDI-formulated catalase particles as evaluated by a twin stage impinger after storage up to 12 weeks.

Formulation	Stage	Fraction at week-1 (%)	Fraction at week-6 (%)	Fraction at week-12 (%)
CO1:0	Device	23.71±2.09	10.00±1.02	ND
	Stage 1	43.31±1.32	88.79±1.29	ND
	Stage 2	32.98±0.90	1.20±2.07	ND
CPT5:1:6	Device	15.84±3.01	13.16±2.58	17.85±1.31
	Stage 1	25.24±1.00	32.47±1.46	30.74±4.77
	Stage 2	58.92±3.02	54.37±3.89	53.26±6.08

The spray-dried catalase formulation containing either sucrose or trehalose as stabiliser, produced a significantly higher stage 2 deposition of protein relative to the MDI CO1:0 formulation, as evaluated during the first week after manufacture ($p<0.05$, two tailed student t-test). The stage 2 fractions of MDI formulated CS1:1 and CT1:1 appeared to increase from 33.0% in the absence of excipient to 39.3 and 44.8% respectively, when sucrose or trehalose were employed. The stage 1 fractions appeared to be almost identical in the absence or presence of excipient. The fine particle fractions

generated by the CS1:1 and CT1:1 MDI formulations appeared to decrease as a function of storage time. The formulation incorporating trehalose emitted a higher fine particle fraction after 6-26 weeks of storage than the similar formulation containing sucrose. For example, after 26 weeks storage at room temperature, the stage 2 fraction of the CS1:1 MDI formulation was 6.0%, relative to the 18.7% emitted from MDI containing the CT1:1 formulation. The reductions in the fine particle fractions were compensated by increases in the stage 1 fractions, whilst the device fractions were consistently found to be about 20% of the recovered dose and independent of formulation and storage time.

The fine particle fraction of the PVA containing MDI formulation was found to be 58.9%, which was significantly higher than that of the MDI formulated CS1:1 or CT1:1 particles ($p<0.05$, two tailed student t-test), whilst the device and stage 1 fractions accounted for only 15.9 and 25.2% of the recovered does respectively, as evaluated during the first week after preparation. After storage for 6 weeks at room temperature, a slight decrease in fine particle fraction was found, albeit not significant ($p>0.05$, one tailed student's t-test). Moreover, after storage for a further 6 weeks, the recovered fine particle fraction appeared to be the same. When catalase was spray-dried in the presence of PVA-trehalose mixture, the resultant MDI formulations appeared always to display a significantly better aerosol performance in comparison to the MDI formulated CS1:1 or CT1:1 particles during storage ($p<0.05$, paired student t-test).

CLAIMS:

1. A formulation of a therapeutic substance suitable for delivery to a patient by a metered dose inhalation device, the substance being in association with a stabilising amount of a glycoside and being formulated in one or more propellants, characterised in that the therapeutic substance is first prepared as a substantially dry powder in the presence of a polyhydroxylated polyalkene, prior to formulation with propellant.

2. A powdered formulation of a therapeutic agent, a glycoside and a polyhydroxylated polyalkene suitable for incorporation with a propellant for dispensing from a metered dose inhaler.

3. A metered dose inhalation device provided with a reservoir comprising a propellant prepared with a therapeutic substance, a glycoside and a polyhydroxylated polyalkene.

4. A formulation or device according to any preceding claim, wherein the propellant is alkane based.

5. A formulation or device according to any preceding claim, wherein the propellant is a haloalkane.

6. A formulation or device according to any preceding claim, wherein the polyhydroxylated polyalkene is polyvinyl alcohol.

7. A formulation or device according to any preceding claim, wherein the propellant is a hydrofluoroalkane, preferably HFA-134a.

8. A formulation or device according to any preceding claim, wherein the glycoside is trehalose, mannitol or sucrose.

9. A formulation or device according to any preceding claim, wherein the therapeutic agent is selected from antibodies, interferon, such as α-interferon, β-interferon and γ-interferon, enzymes such as proteases and ribonucleases, especially DNase I, hormones, such as insulin, LHRH, granulocyte-colony stimulating factor,

calcitonin, heparin, human growth hormone, leuprolide acetate and parathyroid hormone, protease inhibitors, such as α1 antitrypsin, as well as beclomethasone dipropionate.

ABSTRACT

METERED DOSE INHALATION PREPARATIONS

Glycosidically stabilised macromolecules, such as proteins and peptides, have substantially greater stability in the presence of HFA's for dispensing from MDI's, when formulated with polyhydroxylated polyalkenes, such as PVA.